628.96909786
A7LD

LABORATORY DETERMINATIONS
OF
ACUTE ORAL LD_{50}
AND
EFFICACY OF BRODIFACOUM
ON THE
RICHARDSON GROUND SQUIRREL,
SPERMOPHILUS RICHARDSONII

by

Steven Baril and Greg Pallister

MONTANA DEPARTMENT OF AGRICULTURE
ENVIRONMENTAL MANAGEMENT DIVISION
1981

TECHNICAL REPORT 81-1

SUMMARY

In a laboratory test of Richardson ground squirrels, the LD_{50} for brodifacoum was determined to be 0.127 mg/kg for males (95% confidence interval of 0.000 to 0.258). Because of a large number of deaths at all concentrations tested, an LD_{50} could not be determined with confidence for females. For males and females combined the LD_{50} was 0.130 mg/kg (95% confidence interval of 0.063 to 0.188). A 3-day laboratory free-choice test using 50 ppm brodifacoum on cereal based pellets resulted in 100% morality of tested squirrels. Of the total diet consumed by ground squirrels during the test, 30.3% was composed of the brodifacoum treated pellets.

Montana State Library
3 0864 1006 8557 0

LABORATORY DETERMINATIONS OF ACUTE ORAL LD50 AND EFFICACY OF BRODIFOCOUM ON THE RICHARDSON GROUND SQUIRREL, SPERMOPHILUS RICHARDSONII.

INTRODUCTION

Ground squirrels are among the most destructive agricultural pests in Montana. In 1973, the loss to agriculture from these pests was estimated at $4.7 million for the Richardson ground squirrel and $1.2 million for the Columbian ground squirrel (Seyler, 1973). Effective control of these pests with presently registered rodenticides has been difficult and with the potential restrictions on strychnine use by the EPA, landowners may have few effective alternatives for control of ground squirrels.

Because of potential cancellations or close regulation of acute toxicants such as compound 1080 and strychnine, the development of new and safer rodenticides for use in ground squirrel control is necessary. Some of the more recently developed anticoagulants are promising because they generally are effective with smaller dosages. In addition, anticoagulants in general pose less danger to non-target organisms and an effective antidote, vitamin K, is readily available.

The acceptance of anticoagulants for use in controlling field rodents has been slow for a variety of reasons. Many require multiple doses, hence reapplication, in order to achieve a toxic response. This feature makes these toxicants objectionable to applicators attempting to control rodents on large acreages because the time and effort involved in baiting are increased. Anticoagulants such as diphacinone and chlorophacinone have provided acceptable field rodent control in

Digitized by the Internet Archive
in 2017 with funding from
Montana State Library

https://archive.org/details/laboratorydeterm1981bari

some areas but require the use of bait stations maintained daily which detracts from their attractiveness for ground squirrel control in Montana. Further, the degree of control obtained by many anticoagulants is generally less than the control achieved by acute poisons.

Brodifacoum, an analog of difenacoum, is a recently developed second generation anticoagulant now marketed in the U.S.A. for commensal rodent control. It also appears to have potential for use in controlling field rodents. The LD_{50} for non-commensal rodents is very low in comparison with other anticoagulants (Dubock and Kaukeinen, 1978) and multiple feedings may not be necessary in order to ingest a lethal dose. In trials using 50 ppm brodifacoum against noncommensal species, Marsh (as cited in Dubock and Kaukeinen, 1978) obtained 100% mortality of the deer mouse, Peromyscus maniculatus, in a 1-day no-choice test, and 100% mortality of the ground squirrel, Spermophilus beecheyi, in 1, 2, or 3-day no-choice tests. And, as with other anticoagulants, vitamin K is the antidote and the toxicity to non-target species may be less than that of some acute rodenticides (Godfrey et al. 1981 a, b).

The following laboratory study was conducted to test brodifacoum using Richardson ground squirrels as test animals to accomplish the following objectives:

1) determine the LD_{50} of technical brodifacoum
2) determine the efficacy of a 50 ppm concentration offered in a free-choice situation

METHODS

Richardson ground squirrels were captured in Lewis and Clark County, Montana, in July 1979 for the LD_{50} determination, and in May 1980 for the efficacy study. In 1980 a high ratio of adult females to adult males precluded capture of equal numbers of each sex. Animals were captured using 6x6x19 inch live traps and were dusted with pyrethrin to remove ectoparasites prior to being housed in 12x9x12 inch metal cages. Standard laboratory rations consisted of Purina Formulab Chow #5008 and water ad lib.

Acute Oral LD_{50} Determination.

Ground squirrels captured for this study consisted of non-lactating adult females, adult males, and 3 month old juveniles. All animals were acclimated to laboratory conditions for at least one week prior to testing. Temperature was maintained at 24° C and photoperiod at 12 hours light per day.

Procedures for determining the acute oral LD_{50} were similar to those recommended for vertebrates by the American Society for Testing and Materials (1978). A prepared solution of technical brodifacoum, 100 mg/l in propylene glycol, was used for preparing solutions. Intubation solutions were prepared by diluting the prepared solution with propylene glycol to make concentrations of 100, 75, 50, 25, and 12.5 mg/l.

Prior to testing, ground squirrels were assigned to 6 groups of 10 animals each, 5 males and 5 females. Only animals that were eating regularly and appeared healthy were tested and they were distributed among the groups on the basis of size in order to minimize variations due to weight.

After the acclimatization period, animals were fasted for 24 hours and then anesthetized with metofane (methoxyflurane) and weighed to the nearest 0.1 gram. Brodifacoum solutions were administered with a number 18 animal feeding needle at the rate of 1 ml. of solution to 100 g. of body weight so that acute oral dosages were as follows: 1.00, 0.75, 0.50, 0.25, and 0.125 mg. per kg. of body weight. The control group received 1 ml. of propylene glycol per 100 gm. of body weight. Some difficulty was encountered during the intubation process and several ground squirrels did not recover. These were considered to have died as a result of intubation.

After gavaging, the test animals were observed for 21 days or until death and toxic responses recorded daily. All animals were frozen at death for subsequent necropsy to determine sites of internal hemorrhage.

The data were evaluated using a probit model to determine the LD_{50} and the associated confidence intervals.

Free Choice Efficacy Study

Richardson ground squirrels used in this portion of the study consisted of adult males and adult lactating or non-lactating females, none of whom were pregnant. Methods were modified to suit ground squirrels from those of Byers and Palmateer (1979) for dry bait laboratory efficacy methods. All animals were acclimated to laboratory conditions for at least one week during which time they were given Purina Formulab Chow #5008 mixed equally with rolled oats. Food consumption was determined for 3 consecutive days prior to testing and only animals that

were eating regularly and appeared healthy were included in the test. The test group consisted of 6 males and 14 females and the control group included 4 males and 6 females. Animals were distributed between the two groups on the basis of weight in order to minimize variations due to size.

The free choice test was conducted for 3 days during which the test animals were given 100 g. each of the lab diet and cereal based pellets containing 50 ppm brodifacoum (Talon^{TM} Rodenticide, ICI Americas, Inc.). Consumption of the treated and standard lab diets was determined to 0.1 g. Spilled and damp food was oven dried for 24 hours prior to weighing. Positions of the food dishes were switched each day to discourage feeding site bias. Ground squirrels were observed for 10 days post-test and toxic responses and food consumption recorded. All test animals succumbed during the observation period.

Body weights were recorded to the nearest 0.1 g. at the start of the test and at death or the end of the observation period. For this procedure, ground squirrels were coaxed into a cloth bag and weighed without the use of anesthesia.

Where doubt existed as to the cause of deaths, ground squirrels were frozen for later necropsy.

RESULTS

Acute Oral LD_{50} Determination

Brodifacoum intubations caused deaths at every concentration that was tested (Table 1 and Appendix A). At 0.125 mg/kg, 62.5%

TABLE 1. Mortality and average changes in body weight following intubations with brodifacoum in Richardson ground squirrels.

Dose (mg/kg)	Mortality	Days to Death Mean	Days to Death Range	Weight Change
Control	0/9	–	–	+38.3
0.125	5/8	12.4*	8-17	-17.9
0.250	9/10	6.6*	3-12	-32.0
0.500	10/10	7.5	5-14	-58.5
0.750	8/8	7.3	5-10	-28.1
1.000	10/10	6.5	4-10	-24.8

*Calculations include only those squirrels that died during the test.

mortality occurred, 90% at 0.250 mg/kg, and 100% mortality occurred at all other doses tested. The acute LD_{50} determined by probit evaluation was 0.125 mg/kg with a 95% confidence interval of 0.000 to 0.258 for males. An LD_{50} could not be determined with confidence for females because of the large number of responses at all doses. For both sexes combined, the LD_{50} was 0.130 with a confidence interval of 0.063 to 0.188 mg/kg.

Upon necropsy, all but two treated ground squirrels had internal or external hemorrhages. These two had survived from the 0.125 and 0.250 mg/kg tests. All other test squirrels appeared to have died from hemorrhages. Parts affected included the liver and gall bladder, heart, caecum, small intestine, stomach, mesenteries, thoracic and abdominal walls, and feet.

Free Choice Efficacy Study

All ground squirrels that were offered the toxic bait died during the study. Deaths began on the third day after treatment and the last squirrels died on the 12th day. There was no apparent difference in the susceptibility of males and females (Table 2).

Of the total food consumed by the treated group during the 3-day choice test, 30.4% was composed of the brodifacoum treated pellets. (Table 2). Total food consumption remained essentially the same before and during the test. Consumption of the control group increased slightly during that time.

TABLE 2. Consumption of treated and untreated food, changes in body weight, and mortality in a laboratory efficacy test with 50 ppm brodifacoum.

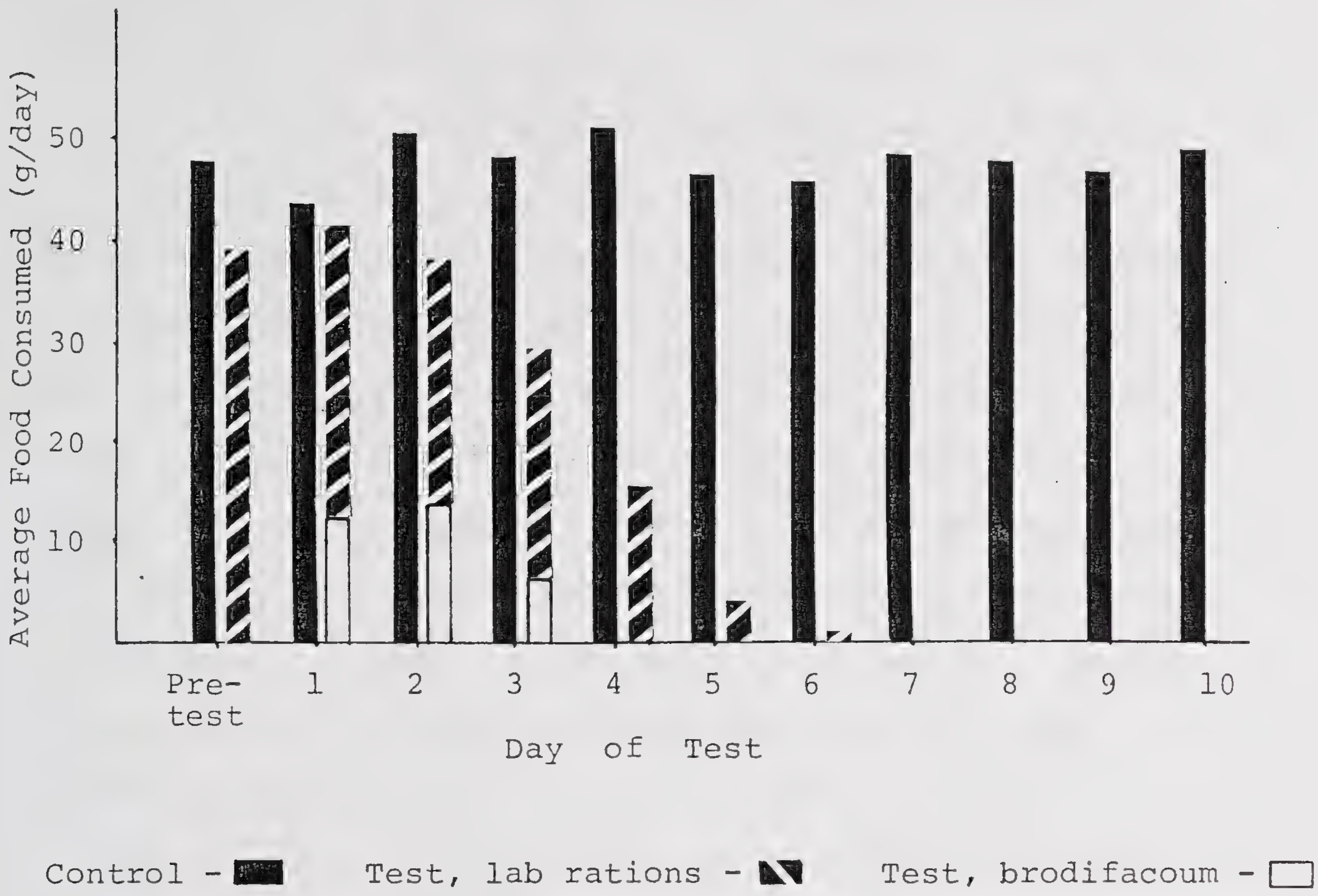
			Average Food Consumed (g/day)					
			PRETEST	TEST				
Group	Sex	Number Tested		Toxic	Untreated	% Toxic	Mean Weight Change	Mean Days to Death
Control	F	6	31.9	-	34.0	-	+67.3	-
	M	4	65.3	-	68.2	-	+142.2	-
	M & F	10	45.3	-	47.6		+97.2	-
Test	F	14	34.3	10.3	22.3	31.6	-25.2	6.50
	M	6	45.8	13.0	33.0	28.3	-15.2	6.00
	M & F	20	37.8	11.1	25.5	30.3	-22.4	6.35

At the start of the test, average total food consumption was similar for control and test groups (Figure 1, Table 2). However, food intake of the treated squirrels began decreasing on the second day of treatment and after the sixth day had stopped.

Males consumed more treated food daily per animal than did females but the proportion of the diet was smaller; 31.6% for females and 28.3% for males. Average active ingredient consumed per day was 0.515 and 0.650 mg for females and males, respectively.

Weight changes were positive for control and negative for treated squirrels. Changes in body weight and food consumption are presented for all test animals in Appendix B.

Figure 1. Average daily consumption of brodifacoum treated pellets versus untreated lab rations during a 3 day choice test on Richardson ground squirrels.



DISCUSSION

The number of deaths that occurred at the lowest doses tested was not anticipated in the LD_{50} study. In future LD_{50} determinations on ground squirrels the range of dosages to be tested should probably be lower than those reported here.

The LD_{50} of 0.130 mg/kg for male and female Richardson ground squirrels combined is low compared with that determined for brodifacoum for other small mammals. The LD_{50} of brodifacoum is 0.36 mg/kg for pine voles and 0.72 mg/kg for meadow voles (Byers, 1978). For commensal rodents, LD_{50}'s are 0.26 for male Rattus norvegicus and 0.40 mg/kg for Mus muscules (Redfern et. al., 1977). Recent information indicates that brodifacoum is a potent toxicant to other mammals. Godfrey et. al. (1981 a,b) has determined that the acute oral LD_{50} to rabbits is 0.20 mg/kg and for dogs, 1.09 to 3.56 mg/kg.

The 100% mortality obtained in the 3-day efficacy test is promising; however, 30.3% acceptance of the brodifacoum pellets suggests that further work on palatability may improve bait acceptance. Minimum requirements for bait acceptance in 3-day tests have not been established for ground squirrels. However, the EPA Animal Biology Laboratory recommends a minimum of 33% acceptance of nonwaxed dry-bait anticoagulants in 15-day choice feeding tests (Peacock and Palmateer, 1979). Alternate carriers, such as oat groats, may improve bait acceptance.

Broadifacoum is a promising chemical for ground squirrel control. For comparison with two acute toxicants now used for

ground squirrel management, the LD_{50} of strychnine is approximately 24 mg/kg for the California ground squirrel. The LD_{50} of compound 1080 is 0.35 mg/kg for the California ground squirrel (Denver Wildlife Research Center Files). If brodifacoum is more toxic than these chemicals, as it appears to be, investigations for field rodent control should continue. Because of the potent toxicity of brodifacoum, investigations should also be undertaken on its toxicity to various wildlife.

LITERATURE CITED

American Society for Testing and Materials. 1978. Standard recommended practice for determining acute oral LD_{50} for testing vertebrate control agents. 1978 Annual Book of ASTM Standards, part 46, ASTM Designation E 555 - 75. pp. 347-348.

Byers, R. E. 1978. Performance of rodenticides for the control of pine voles in orchards. J. Amer. Soc. Hort. Sci. 103: 65-69.

Byers, R. E. and S. C. Palmateer. 1979. Microtus dry bait laboratory efficacy test method. Pages 101-106 in: J. R. Beck, Ed. Vertebrate Pest Control and Management Materials. ASTMSTP 680, American Society for Testing and Materials, Philadelphia, PA.

Denver Wildlife Research Center Files. As cited in U.S. Environmental Protection Agency. 1980. Strychnine Postion Document 2/3. Office of Pesticide Programs, Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency. 110 pp.

Dubock, A. C. and E. E. Kaukeinen. 1978. Brodifacoum (Talon^{TM} Rodenticide), a novel concept. Pages 127-137 in: W. E. Howard and R. E. March, Eds. Proceedings Eighth Vertebrate Pest Conference. University of California, Davis, CA.

Godfrey, M. E. R., T. C. Reid, and H. J. F. McAllum. 1981 a. The oral toxicity of brodifacoum to rabbits. N. Z. Journal Exp. Agric. 9:23-25.

Godfrey, M. E. R., T. C. Reid, and H. J. F. McAllum. 1981 b. The acute oral toxicity of the anticoagulant broadifacoum to dogs. N. Z. Journal Exp. Agric. 9:147-149.

Peacock, D. B. and S. C. Palmater. 1979. Comparison of EPA Animal Biology Laboratory and company laboratory efficacy data for federally registered rat and mouse baits. Pages 11-19 in: J. R. Beck, Ed. Vertebrate Pest Control and Management Materials. ASTMSTP 680, American Society for Testing and Materials, Philadelphia, PA.

Redfern, F., J. E. Gill, and M. R. Hadler. 1977. Laboratory evaluation of WBA 8119 as a rodenticide for use against warfarin-resistant and non-resistant rats and mice. J. Hyg., Camb. 77:419-426.

Seyler, K. 1973. Rodent damage and control survey of 50 Montana counties. Montana Department of Livestock report, Helena, MT. 3 pp.

Appendix A. Mortality and changes in body weight caused by intubations with brodifacoum in Richardson's ground squirrels.

Treatment	Sex	Initial Weight (g)	Weight Change	Days to Death
Control (1 ml propylene glycol/ kg)	M	393.0	+55.9	-
	M	378.4	-51.0	0 *
	M	357.7	+11.8	-
	M	344.8	+11.3	-
	M	328.8	+31.2	-
	F	356.7	+ 5.9	-
	F	332.0	+66.0	-
	F	308.9	+51.9	-
	F	307.0	+42.7	-
	F	284.6	+67.8	-
0.125 mg/kg	M	326.1	-44.8	0 *
	M	300.3	-65.2	15
	M	295.5	-136.6	17
	M	291.7	+178.8	-
	M	358.5	-51.1	8
	F	230.0	-59.7	13
	F	414.7	-40.7	-
	F	411.1	-49.1	9
	F	273.3	-16.3	0 *
	F	266.8	+81.0	-
0.250 mg/kg	M	383.0	-22.1	7
	M	374.8	+33.2	-
	M	354.9	-30.6	5
	M	344.5	-23.7	5
	M	338.2	-60.9	5
	F	220.9	- 3.7	3
	F	320.9	-100.9	8
	F	310.9	-54.9	12
	F	298.6	-42.5	8
	F	291.4	-13.9	6
0.500 mg/kg	M	424.1	-12.4	14
	M	364.0	-37.7	7
	M	353.5	-61.7	6
	M	344.5	+ 2.3	6
	M	344.2	- 4.0	6
	F	370.0	-142.9	7
	F	327.2	-56.8	7
	F	313.4	-90.0	11
	F	301.8	-71.7	6
	F	286.4	+ 2.1	5
0.750 mg/kg	M	393.0	-37.3	9
	M	360.8	+15.7	7
	M	347.7	-79.3	7
	M	344.2	-19.6	0 *
	M	340.3	-55.3	9
	F	334.9	-17.2	5
	F	325.2	- 3.8	6
	F	308.6	-19.8	5
	F	300.0	-25.4	0 *
	F	294.0	-27.7	10
1.000 mg/kg	M	391.3	-20.6	10
	M	361.5	-31.6	7
	M	346.4	-16.4	6
	M	345.5	-30.5	5
	M	328.2	-30.7	7
	F	336.0	-23.7	7
	F	330.0	-48.7	7
	F	309.6	-28.7	4
	F	297.3	+ 6.7	5
	F	281.9	-23.2	7

* Did not recover following intubation

Appendix B. Initial and final body weights and consumption of untreated and brodifacoum treated food for a 3-day choice test using Richardson's ground squirrels.

Animal No.	Sex	Initial Weight (g)	Final Weight	Pretest Diet (g/day)	Test Period Diet			Days to Death
					Toxic	Untreated	Total	
1	F	287.1	351.2	23.3	-	30.0	30.0	-
2	M	467.8	627.1	62.0	-	63.7	63.7	-
3	F	338.7	379.2	32.0	-	26.4	26.4	-
4	M	566.3	751.1	79.0	-	76.2	76.2	-
5	M	480.7	662.5	67.7	-	70.6	70.6	-
6	F	344.6	436.2	31.7	-	40.2	40.2	-
7	F	296.4	355.4	29.7	-	31.4	31.4	-
8	F	262.3	334.9	56.0	-	44.7	44.7	-
9	M	577.0	620.0	52.3	-	62.1	62.1	-
10	F	241.6	317.3	19.0	-	31.0	31.0	-
11	F	321.5	259.6	26.3	10.6	17.6	28.2	11
12	M	355.2	340.2	24.7	9.6	13.7	23.3	6
13	M	490.4	494.2	70.0	19.9	47.4	67.3	5
14	F	366.3	361.1	45.3	11.3	30.7	42.0	4
15	M	637.1	593.1	38.3	14.9	37.8	52.7	10
16	F	422.8	421.6	53.3	9.7	23.8	33.5	2
17	F	312.3	318.0	29.0	11.0	18.3	29.3	2
18	F	441.9	383.9	32.3	12.6	36.6	49.2	9
19	F	306.8	277.9	35.7	6.3	21.1	27.4	4
20	F	277.0	284.9	25.3	7.3	21.9	29.2	7
21	M	608.8	566.2	73.0	6.7	36.7	43.4	6
22	F	368.2	311.6	40.0	11.2	21.2	32.4	11
23	F	303.1	301.2	27.3	15.9	16.1	32.0	4
24	F	265.7	237.1	30.0	5.8	20.3	26.1	5
25	F	329.3	274.0	34.7	12.8	20.9	33.7	9
26	F	369.2	349.8	43.7	11.5	27.6	39.1	6
27	F	321.7	296.4	27.3	10.3	17.4	27.7	11
28	M	452.1	445.7	22.0	8.3	18.6	26.9	3
29	F	250.0	221.7	30.0	8.2	18.6	26.8	6
30	M	483.0	496.1	46.7	18.5	43.9	62.4	6